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Two new records of *Agaricus* spp. from Ethiopia

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Abstract — *Agaricus xanthodermus* and *A. xanthodermulus* are recorded as new to Ethiopia based on morphological and molecular data. A description of *A. campestris* (previously reported from Ethiopia) is also provided. The specimens were collected from Menagesha and Holeta sites located in the Wolmera District of Oromia Special Zone surrounding Addis Ababa. Morphological identification of the specimens was supported by nrDNA ITS sequence analysis. All three species are described in detail and illustrated based on the collections from Ethiopia.

Key words — *Agaricaceae*, *Basidiomycota,* taxonomy, highland, mycota

Introduction

Agaricus L. is a large and important genus encompassing many edible and few poisonous mushrooms, with possibly 200–400 (Bas 1991, Kirk et al. 2008, Zhao et al. 2011) to possibly over 400 (Thongklang et al. 2014) species worldwide. *Agaricus* species are generally distinguished from other members of the family *Agaricaceae* by their small to large basidiomata, white or brown pileus, annulus and central stipe, free lamellae that are whitish or pinkish when young and chocolate-brown to dark brown when mature, and smooth dark basidiospores (Cappelli 1984). Studies have shown that the genus is monophyletic (Geml et al. 2004) and contains eight recognized sections (Parra 2008), some of which have been phylogenetically reconstructed, e.g., *A.* sect. *Bivelares* (Kauffman) L.A. Parra and *A.* sect. *Xanthodermatei* Singer (Challen et al. 2003, Kerrigan et al. 2005, Thongklang et al. 2014). *Agaricus* species are generally found in pastures among grass and in mixed forests (Bas 1991).

Table 1. ITS sequences of *Agaricus* and outgroup species used in the molecular analysis.

Species	Voucher/strain	Origin	GenBank*
Agaricus aff. *endoxanthus*	ZRL3095	Thailand	JF691554
A. aridicola	CA101	France	JF797195
A. arvensis	CA640	France	JF797194
A. augustus	CA590	France	JF797193
A. benesii	LAPAG283	France	JF797179
A. bernardii	strain ARP173	USA	AF432880
A. bitorquis	strain RWK1462	USA	AF432898
A. bohusii	LAPAG531	Czech Republic	JF797180
A. bresadolanus	strain CA177	France	DQ185570
A. brunneolus	CA490	France	JF797203
A. californicus	strain RWK 1936	USA	DQ182510
A. campestris	strain W1H	USA	DQ182533
	Strain B9	China	JX434655
	strain H2	India	KM609406
	strain AGRK111	China	FJ223222
	HMAS272461	Ethiopia, Menagesha	**KP229419**
	HMAS272462	Ethiopia, Holeta	**KP229420**
A. caribaeus	F2530	Martinique	JF727856
A. cupreobrunneus	LAPAG 322	Spain	JQ824136
	strain CA 87	USA	DQ182532
A. fissuratus	strain WC777	Denmark	AY484683
A. gennadii	CA387	France	JF797188
A. langei	strain WC784	USA	AY484699
	LAPAG141	France	JF797181
A. laskibarii	LAPAG115	USA	*AY943975*
A. moelleri	strain CA31	France	AY899263
	strain CA156	France	AY899264
A. parvitigrinus	strain CA157	France	AY899266
	strain CA158	France	AY899267
A. pseudolutosus	LAPAG77	Spain	JF727868
A. silvaticus	LAPAG341	France	JF797178
A. viridopurpurascens	Horak68/79	New Zealand	JF514525
A. xanthodermus	strain CA15	France	AY899271
	strain CA161	France	AY899272
	strain W3I	England	DQ182534
	strain CA236	France	DQ185564
	strain CA6	France	DQ185563
	HMAS272456	Ethiopia, Menagesha	**KP229414**
	HMAS272457	Ethiopia, Holeta	**KP229415**
A. xanthodermulus	strain CA160	France	AY899273
	strain CA174	France	AY899274
	strain CA204	France	AY899276

Table 1, concluded

(*A. xanthodermulus*)	LIP CA160	France	NR119528
	strain CA188	France	AY899275
	HMAS272459	Ethiopia, Holeta	**KP229416**
	HMAS272460	Ethiopia, Menagesha	**KP229417**
A. xanthosarcus	Goossens5415	Belgium	JF514523
Hymenagaricus ardosiicolor	LAPAF9	Togo	JF727840
Heinemannomyces splendidissimus	ecv3586	Thailand	HM488760

* Sequences produced in this study in bold.

Pegler (1977) noted that agaric mycota in East Africa is extremely rich both in the range of genera and in the number of species. However, large parts of this region remain mycologically unexplored. Although Ethiopia is known to have highly diverse flora and fauna (Friis & Sebsebe 2001) but the diversity, ecology, and distribution of macrofungi is so far poorly explored.

Macrofungal diversity in general and *Agaricus* species in particular have not been a focus of taxonomic investigation in Ethiopia. Only a few reports on macrofungi in Ethiopia have been published (Abate 1995, 1999). Abate (1999), who cited *Agaricus campestris* as the dominant mushroom in the grazing areas on the highland (2000–3000 m) plateau in southern (Bale), central (Debreberhan), and northwestern (Gojam) Ethiopia, appearing in a large numbers during the middle of the rainy season (July and August) did not provide a detailed morphological description or any molecular data of the species.

As the first report of the on-going work on macrofungal diversity in Ethiopia, we present detailed morphological descriptions and molecular analyses of three *Agaricus* species including two new records, *A. xanthodermus* and *A. xanthodermulus,* for Ethiopia in addition to the previously cited *A. campestris*. This report will provide a baseline for future taxonomic, ecological, and economic studies on Ethiopian *Agaricus* species.

Materials & methods

Collection sites

Specimens were collected from Menagesha and Holeta, Wolmera district, in the Oromia Special Zone surrounding Addis Ababa, the capital of Ethiopia, during June–September, 2012–2013. Menagesha (9°02′N 38°35′E, 2500–3300 m a.s.l.) comprises forests and grazing areas. Holeta (9°03′N 38°30′E, 2391 m a.s.l.) contains mainly settlement and grazing areas.

Morphological observation

Distinguishing macroscopic features and microhabitat were noted for each specimen in the field. Microscopical observations were made from dried specimens mounted in 5% KOH and stained with 1% aqueous Melzer's reagent and Congo red. At least 20

basidiospores were measured from each specimen. Chemical testing involved the application of 20% KOH on the pileus surface, with negative = no colour change and positive = change to yellow or orange. Dried specimens are deposited in the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia, and duplicates are preserved in the Fungarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). The specimens were identified morphologically according to Pegler (1977), Pegler & Piearce (1980), Singer (1986), and Callac & Guinberteau (2005). Abbreviations: RSK = Rediet Sitotaw Kebede.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from dried specimens using the modified CTAB method described by Yao et al. (1999). The fungal universal primer pairs ITS5/ITS4 were used to amplify the internal transcribed spacer (ITS) region and LR0R/LR5 to amplify the nuclear larger subunit RNA (LSU) region (White et al. 1990). The PCR reaction mixture was comprised 25 µL Taq PCR MasterMix, 0.5 µL of 10 µM each primer, 1 µL diluted DNA template, and RNase-Free water to bring the total volume to 50 µL. The PCR conditions were as follows: 94 °C for 5 min, followed by 35 cycles of 95 °C for 1min, 53 °C for 1min, 72 °C for 1 min and final extension step of 72 °C for 10 min on a GenAmp PCR System 9700 thermocycler (Vers. 3.03).

PCR products were sequenced from both directions using the same primers on an Applied Biosystems 3730 Analyzer™ by the Beijing Genomics Institute (Beijing, China). Sequence ends were manually edited and assembled using DNAStar Lasergene SeqMan™ II vers. 6.1. Sequences were then aligned using Clustal W algorithm (Thompson et al. 1994) and manually edited with BioEdit vers. 7.1.9 (Hall 1999).

Phylogenetic analysis

ITS sequences from the Ethiopian *Agaricus* collections were aligned with 43 closely related ITS sequences from GenBank representing the eight *Agaricus* sections (Zhao et al. 2011) (Table 1). *Hymenagaricus ardosiicolor* and *Heinemannomyces splendidissimus* were selected as outgroup taxa since these species consistently formed a sister clade to the monophyletic *Agaricus* (Zhao et al. 2011). The datasets were analysed using PAUP vers. 4.0b8 (Swofford 2002). Maximum parsimony (MP) analyses were conducted with 1000 replicates of random sequence addition followed by tree-bisection reconnection branch swapping. All characters were equally weighted, and gaps were treated as missing data. Bootstrap proportions (BP) were calculated using analyses of 1000 replicates with five replicates of random sequence addition.

Results of phylogenetic analyses

ITS sequences from the six Ethiopian specimens were submitted to GenBank as KP229414–229417, 229419, and 229420. (Analysis of the LSU sequences also obtained from the same specimens and deposited in GenBank as KP331526–KP331530 will be presented in a future publication.)

A total of 49 ITS sequences (Table 1) were aligned for the phylogenetic analysis. The dataset generated a MP tree with 709 total characters, of which 439 were constant and 185 parsimony informative sites. Our ITS sequence analysis

supported the eight major *Agaricus* sections, and MP analyses confirmed the placement of our six sequences in two well-defined sections (Figure 1).

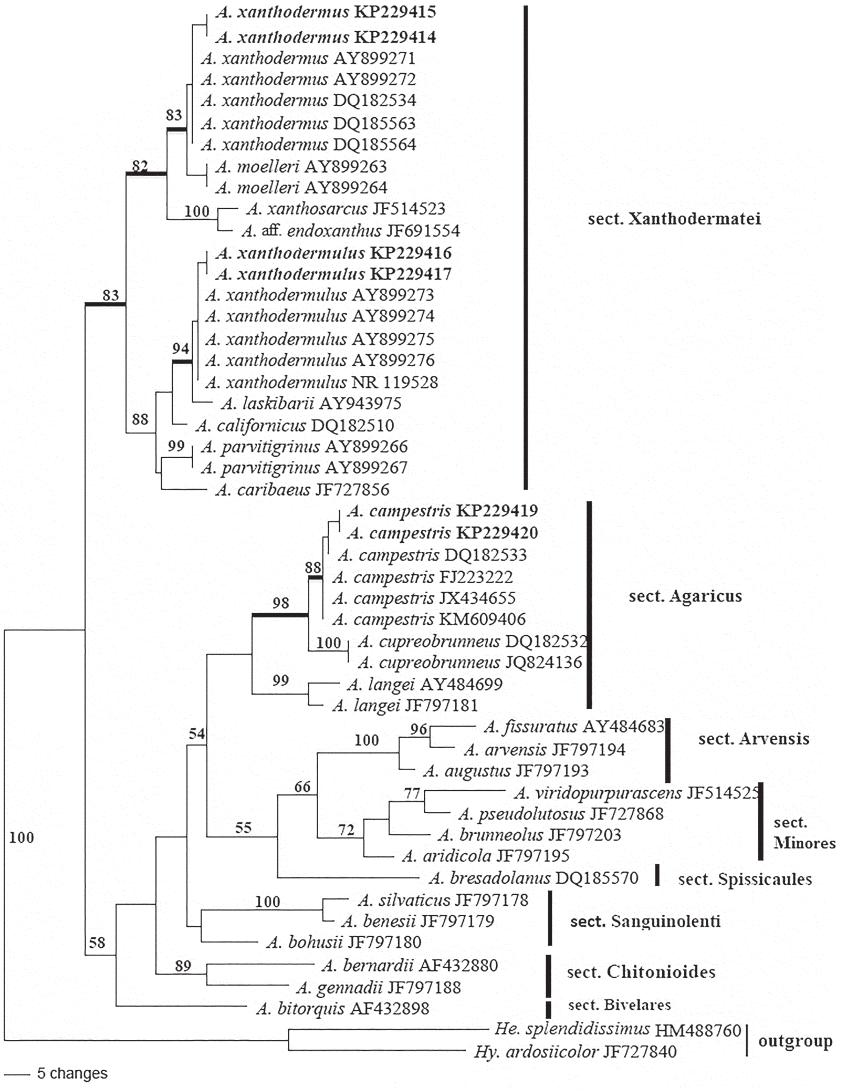

Figure 1. MP tree based on nrDNA ITS sequences. The species in bold were sequenced by the authors. Bootstrap numbers greater than 50% are included above branches; analysis performed in PAUP* using 1000 bootstrap replicates.

Two sequences (KP229419, KP229420) fell within *A.* sect. *Agaricus*, where they grouped with *A. campestris* sequences retrieved from GenBank with 88% bootstrap support. The other four sequences fell within *A.* sect. *Xanthodermatei,* two (KP229416, KP229417) grouping with *A. xanthodermulus* with 94% bootstrap support for the clade (containing *A. xanthodermulus* and *A. laskibarii*) and two (KP229414, KP229415) grouping with *A. xanthodermus* with 83% bootstrap support for the clade (containing *A. xanthodermus* and *A. moelleri*).

Taxonomy

Agaricus campestris L., Sp. Pl. 2: 1173 (1753). Fig. 2

Pileus 4–8 cm diam., at first subglobose to hemispherical, becoming convex, broadly convex to plano-convex, sometimes slightly umbonate; surface white to cream-color, sometimes slightly ochraceous, with grayish to pale ochraceous fibrils or fibrillose scales, dry, sometimes silky; margin incurved at first, finally expanding to decurved, with remnants of partial veil attached. Lamellae free, ≤5 mm wide, initially pinkish, then pinkish brown to cinnamon-brown, finally dark brown, crowded, with lamellulae. Stipe 3–8 × 0.5–1(–1.5) cm, central, cylindrical or slightly tapering toward base; surface white and silky above the annulus, white to brownish with whitish appressed fibrils below; solid to soft, fibrous. Context white or whitish, fleshy, soft. Annulus thin, white, membranous, superior. Odor and taste pleasant. Basidiospore deposit dark brown. Chemical reaction 20% KOH negative.

Basidiospores 5–8.5 × 3.5–5.5 µm, avl × avw = 6.5 × 4.5 µm, Q = 1.4–1.5, avQ = 1.45, ellipsoid to ovoid, pale brown to brown, inamyloid, smooth, thick-walled. Basidia 18–24 × 6–8 µm, clavate, 4-spored, thin-walled, subhyaline to brownish. Lamella edge heterogeneous, with sterile cells, 14–22 × 5–7.5 µm, clavate, thin-walled, subhyaline to brownish. Cheilocystidia not found. Subhymenium ≤10 µm wide; hyphae narrow, branched, hyaline, thin-walled, 2–5 µm diam. Hymenophoral trama regular, 80–120 µm wide, hyphae hyaline, thin-walled, 4.0–20 µm diam. Pileipellis a radially aligned repent epicutis; hyphae 3.5–10 µm diam., cylindrical, branched, hyaline to subhyaline, thin-walled. Pileus trama hyphae 4.5–20 µm diam., cylindrical to inflated, hyaline, thin-walled. Annulus hyphae 3.5–8.5 µm diam., cylindrical, hyaline to subhyaline, thin-walled. Clamp connections absent.

Habitat: Scattered in pastureland.

Specimens examined: **ETHIOPIA**, **Oromia Special Zone**, **Wolmera District**, Holeta, on grazing land, 26 June 2012, RSK32 (HMAS 272461; GenBank KP229419); Menagesha, Menagesha suba forest, 10 July 2013, RSK33 (HMAS 272462; GenBank KP229420).

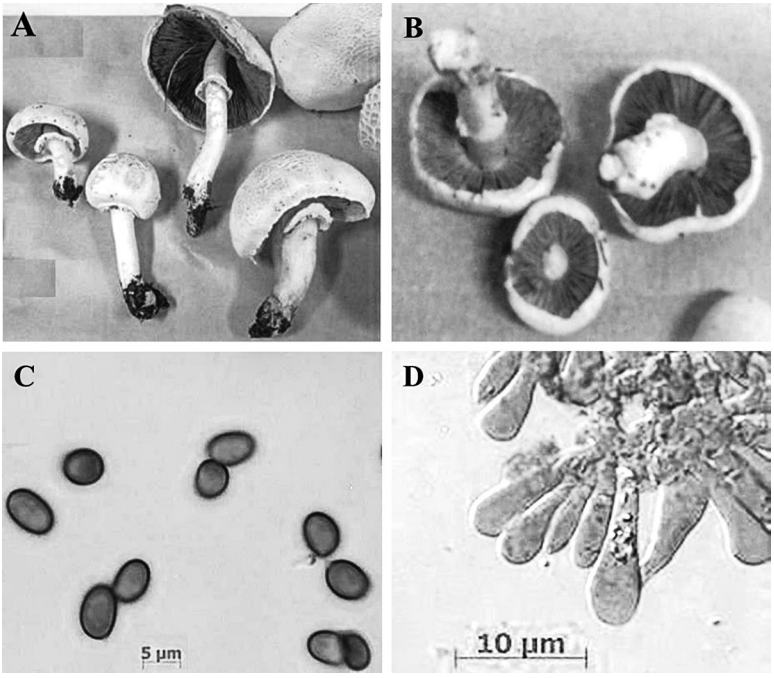

Figure 2. *Agaricus campestris*:
A. basidiomata (HMAS 272461); B. basidiomata (HMAS 272462);
C. basidiospores (HMAS 272461); D. cystidia (HMAS 272462).

Remarks—Although *A. campestris* was first reported from Ethiopia by Abate (1999), we have included this species to provide detailed morphological and molecular data from Ethiopian specimens. As noted by Abate (1999), *A. campestris* was very common on the grazing area of the highland plateau of Ethiopia and we found it to be fairly common and widespread in the grazing area (above 2300 m) right after the beginning of the rainy season. Our *A. campestris* specimens share similar morphological traits of the *A. campestris* complex: a white, subglobose to broadly convex pileus, initially pinkish then pinkish brown and finally dark brown lamellae, a membranous white annulus, a negative KOH reaction, and a mild taste and odor (Pegler 1977, Mitchell & Walter 1999, Kerrigan et al. 2005).

Agaricus xanthodermulus Callac & Guinb., Mycologia 97: 421 (2005). Fig. 3

Pileus 5–7 cm diam., cuboidal at first, then conico-convex to broadly convex, finally expanding to plano-convex, slightly umbonate at center; surface

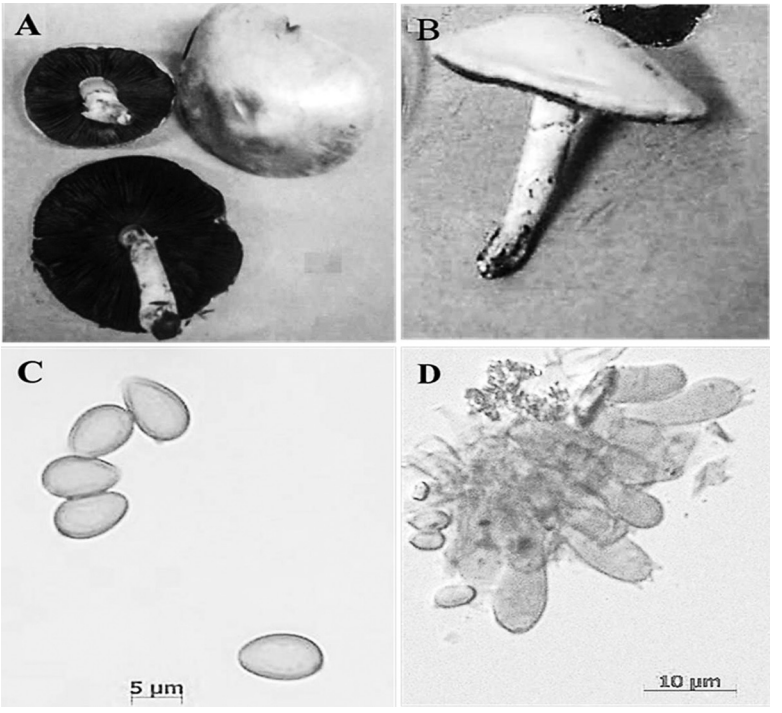

Figure 3. *Agaricus xanthodermulus*:
A. basidiomata (HMAS 272459); B. basidiomata (HMAS 272460);
C. basidiospores (HMAS 272459); D. basidia (HMAS 272460).

white to grayish, becoming yellow when rubbed, sometimes squamulate, dry; margin incurved at first, then decurved. Lamellae free, ≤5 mm wide, pinkish at first, then pinkish brown to brown, finally dark brown, crowded, with lamellulae. Stipe 4–8 × 0.5–1 cm, central, cylindrical with slightly bulbous base; surface white to whitish, becoming yellow when rubbed; solid at first, then soft, finally hollow, and fibrous in old specimen. Context white or whitish, becoming light yellow when cut, fleshy. Annulus white to whitish, membranous, superior. Basidiospore deposit dark brown. Taste and Odor unpleasant. Chemical reaction with 20% KOH positive.

Basidiospores 5–7 × 4–5.5 µm, avl × avw = 6.0 × 4.7 µm, Q = 1.25– 1.27, avQ = 1.26 ellipsoid, pale brown to brown, inamyloid, smooth, thick-walled. Basidia 18–27 × 6–8 µm, clavate, 4-spored, thin-walled, subhyaline to brownish. Lamella edge heterogeneous, with sterile cells, 15–24 × 5–7 µm, clavate, thin-walled, subhyaline to brownish. Cheilocystidia rare,

18–27 × 7–11 µm, clavate to pyriform, thin-walled, hyaline. Subhymenium ≤12 µm wide; hyphae narrow, branched, hyaline, thin-walled, 3.5–5 µm diam. Hymenophoral trama regular, 80–120 µm wide; hyphae hyaline, thin-walled, 5–20 µm diam. Pileipellis a repent radially aligned epicutis; hyphae thin-walled, 4–10 µm diam., cylindrical, branched, hyaline to subhyaline. Pileus trama hyphae 5–20 µm diam., cylindrical to inflated, hyaline, thin-walled. Annulus hyphae 4–9 µm diam., cylindrical, hyaline to subhyaline, thin-walled. Clamp connections absent.

Habitat: Solitary, grazing land.

Specimens examined: **ETHIOPIA**, **Oromia Special Zone**, **Wolmera District**, Holeta, on grazing land, 10 July 2013, RSK12 (HMAS 272459; GenBank KP229416); Menagesha, Menagesha suba forest, 10 July 2013, RSK12 (HMAS 272460; GenBank KP229418).

Remarks—*Agaricus xanthodermulus,* described recently by Callac & Guinberteau (2005) from France, differs from other members in *A*. sect. *Xanthodermatei* by its small size (maximum cap diameter 6 cm) and larger spores (6.7–7.8 × 4.5–5.5 µm). The Ethiopian material generally agreed with the original description, although its slightly larger pileus and slightly smaller spores brought it closer to the type specimen of *A. xanthodermus* (with a 6.0–12 cm diam. pileus and 4.0–5.5 × 3.0–4.0 µm spores; Callac & Guinberteau 2005). Kerrigan et al. (2005) suggested that *A. xanthodermulus* might represent a small form of *A. xanthodermus.* The ITS sequences show only two nucleotide differences (at positions 120 and 537) between our collections and *A. xanthodermulus* from France.

Agaricus xanthodermus Genev., Bull. Soc. Bot. Fr. 23: 32 (1876) Fig. 4

Pileus 6–12 cm diam., at first subglobose or hemispherical to campanulate then expanding to convex or broadly convex to finally almost applanate; surface at first whitish to grayish, then grayish to pale grayish brown and grayish brown at center, becoming yellow when rubbed, with pale gray to grayish brown fibrillose squamules, dry; margin incurved at first, then decurved, finally sometimes undulating; with remnants of partial veil attached. Lamellae free, ≤4 mm wide, at first white to pinkish, then pinkish brown to brown, finally dark brown; crowded, with lamellulae. Stipe 8–13 × 0.5–1.3 cm, central, cylindrical with bulbous base; surface white to whitish, and brownish at base, becoming yellow when rubbed, with whitish to brownish fibrils at the lower part; solid to soft, fibrous. Context white or whitish, becoming yellowish to yellow when cut, fleshy, firm. Annulus white to whitish, thin, membranous, superior. Basidiospore deposit chocolate brown to blackish brown. Taste and Odor unpleasant. Chemical reaction with 20% KOH positive.

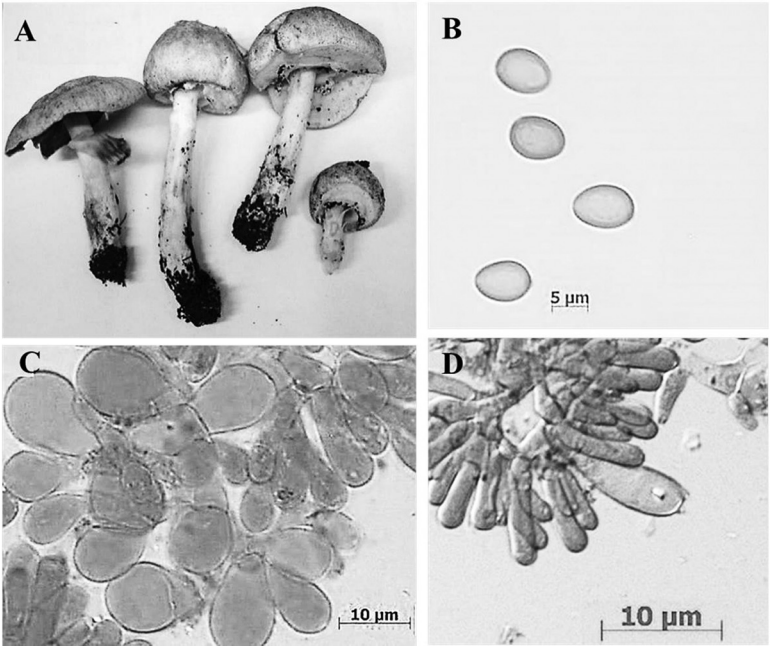

Figure 4. *Agaricus xanthodermus* (HMAS 272456):
A. basidiomata; B. basidiospores; C. cheilocystidia; D. basidia.

Basidiospores 4–5.5 × 3–4 µm, avl × avw = 4.5 × 3.5 µm, Q = 1.3–1.4, avQ = 1.35 ellipsoid, pale brown to brown, smooth, thick-walled, inamyloid. Basidia 15–25 × 6–8 µm, clavate, 4-spored, thin-walled, subhyaline to brownish. Lamella edge heterogeneous, with sterile cells, 14–22 × 4–7 µm, clavate, thin-walled, subhyaline to brownish. Cheilocystidia rare, 18–30 × 6–11 µm, pyriform, thin-walled, hyaline. Subhymenium ≤12 µm wide; hyphae narrow, branched, hyaline, thin-walled, 2.5–5 µm diam. Hymenophoral trama regular, 100–120 µm wide; hyphae hyaline, thin-walled, 4–25 µm diam. Pileipellis a radially aligned repent epicutis; hyphae thin-walled, 4.0–10 µm diam., cylindrical, branched, hyaline to subhyaline. Pileus trama hyphae 5–20 µm diam., cylindrical to inflated, hyaline, thin-walled. Annulus hyphae 4–10 µm diam., cylindrical, hyaline to subhyaline, thin-walled. Clamp connections absent.

Habitat: Growing in groups on grazing land and on soil with plant litter; in semi-open middle-aged mixed forest.

Specimens examined: **ETHIOPIA**, **Oromia Special Zone**, **Wolmera District**, Holeta, on grazing land, 10 July 2013, RSK5 (HMAS 272456; GenBank KP229414); Menagesha, Menagesha suba forest, 10 July 2013, RSK7 (HMAS 272457; GenBank KP229415).

Remarks—*Agaricus xanthodermus* is characterized by its strong and rapid yellow coloration when cut, phenolic odor, cylindrical stipe (often with a small basal bulb), negative Schäffer reaction, and positive KOH reaction. Both macro- and micro-morphological characters observed in the Ethiopian collections were similar to those in the original description. The fungus was common and abundant on grazing land and on litter in semi-open mixed forest in the study sites starting from end of June to the beginning of August. However, ITS sequence analyses showed the clade including the Ethiopian sequences with GenBank sequences of *A. xanthodermus* and *A. moelleri* had only 83% support (Fig. 1). Comparisons of ITS sequences between our collections and *A. xanthodermus* from GenBank indicated three nucleotide differences (at positions 334, 630, and 665). More extensive analyses may reveal the true link among the sequences within the cluster.

Discussion

Morphological observation and molecular analysis placed our Ethiopian collections within two sections, *Agaricus* sect. *Agaricus* and *A.* sect. *Xanthodermatei*.

Agaricus sect. *Xanthodermatei*, represents a monophyletic clade comprising species allied to the type species of the section, *A. xanthodermus* (Kerrigan et al. 2005), known to produce one or more toxic compounds (Wood et al. 1998, Jovel et al. 1996) that can cause gastrointestinal distress leading to violent vomiting. Species in the section are generally considered inedible or not fit for human consumption. The toxicity experienced in Ethiopia after ingestion is reflected by 'Dem Astefy,' the local Amharic name for these mushrooms meaning 'causes vomiting of blood.'

In contrast, the type species of *A.* sect. *Agaricus*, *A. campestris,* is an excellent edible mushroom and abundant at the beginning of the rainy season, but the edibility of this mushroom is not recognized within the Ethiopian community. This might be due to its similarity to the toxic species in *A.* sect. *Xanthodermatei*. The confusability of the mushrooms could lead to mushroom poisoning, leading the local community to shun all the mushrooms growing in the wild and become mycophobic. This in spite of the fact that species of *A.* sect *Xanthodermatei* can usually be distinguished from similar mushrooms (like *A. campestris*) by their tendency to stain and bruise yellow and by the characteristic phenolic odor.

The high *Agaricus* species diversity of *Agaricus* underscores the need for further study of the genus; we hope that many more reports on *Agaricus* species will follow.

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Literature cited

Abate D. 1995. Cultivation of the oyster mushroom in traditional brick pots. Mycologist 9: 179–181. http://dx.doi.org/10.1016/S0269-915X(09)80017-7

Abate D. 1999. *Agaricus campestris* in upland Ethiopia. Mycologist 13: 28. http://dx.doi.org/10.1016/S0269-915X(99)80075-5

Bas C. 1991. A short introduction to the ecology, taxonomy and nomenclature of the genus *Agaricus*. 21–24, in: LJLD van Griensven (ed.). Genetics and breeding of *Agaricus*; proceedings of the First International Seminar on Mushroom Science. Pudoc, Wageningen.

Callac P, Guinberteau J. 2005. Morphological and molecular characterization of two novel species of *Agaricus* section *Xanthodermatei*. Mycologia 97: 416–424. http://dx.doi.org/10.3852/mycologia.97.2.416

Cappelli A. 1984. *Agaricus* L.: Fr. ss. Karsten (*Psalliota* Fr.). Libreria Editrice Biella Giovanna, Saronno.

Challen MP, Kerrigan RW, Callac P. 2003. A phylogenetic reconstruction and emendation of *Agaricus* section *Duploannulatae*. Mycologia 95: 61–73. http://dx.doi.org/10.2307/3761962

Friis IB, Sebsebe D. 2001. Vegetation maps of Ethiopia and Eritrea. A review of existing maps and the need for a new map for the flora of Ethiopia and Eritrea. 399–439, in: Biodiversity Research in the Horn of Africa Region. Proceedings of the 3rd International symposium on the Flora of Ethiopia and Eritrea at the Carlsberg Academy, Copenhagen.

Geml J, Geiser DM, Royse DJ. 2004. Molecular evolution of *Agaricus* species based on ITS and LSU rDNA sequences. Mycological Progress 3: 157–176. http://dx.doi.org/10.1007/s11557-006-0086-8

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium series. 41: 95– 98.

Jovel EM, Towers GH, Kroeger P. 1996. Hydroquinone: the toxic compound of *Agaricus hondensis*. Planta Medica 2(62): 185.

Kerrigan RW, Callac P, Guinberteau J, Challen MP, Parra LA. 2005. *Agaricus* section *Xanthodermatei*: a phylogenetic reconstruction with commentary on taxa. Mycologia 97: 1292–1315. http://dx.doi.org/10.3852/mycologia.97.6.1292

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Dictionary of the fungi, 10th ed. Wallingford, UK: CABI.

Mitchell AD, Walter M. 1999. Species of *Agaricus* occurring in New Zealand. New Zealand Journal of Botany 37: 715– 725. http://dx.doi.org/10.1080/0028825X.1999.9512665

Parra LA. 2008. *Agaricus* L. *Allopsalliota* Nauta & Bas. Part 1. Fungi Europaei, vol. 1. Edizioni Candusso, Alassio, Italy.

Pegler DN. 1977. A preliminary agaric flora of East Africa. Kew Bulletin Additional Series 6. 615 p.

Pegler DN, Piearce GD. 1980. The edible mushrooms of Zambia. Kew Bulletin 35: 475–491.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th ed. Koeltz Scientific Books, Koenigstein, Germany.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associated, Sunderland, MA.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position, specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680. http://dx.doi.org/10.1093/nar/22.22.4673

Thongklang N, Nawaz R, Khalid AN, Chen J, Hyde K, D. Zhao RL, Parra LA, Hanif M, Moinard M, Callac P. 2014. Morphological and molecular characterization of three *Agaricus* species from tropical Asia (Pakistan, Thailand) reveals a new group in section *Xanthodermatei*. Mycologia 106: 1220–1232. http://dx.doi.org/10.3852/14-076

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in MA Innis et al. (eds.), PCR Protocols: a guide to methods and applications. San Diego, Academic Press. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Wood WF, Watson RL, Largent DL. 1998. Phenol, the odour compound from *Agaricus praeclaresquamosus*. Biochemical Systematics and Ecology 26: 793–794. http://dx.doi.org/10.1016/S0305-1978(98)00058-1

Yao YJ, Pegler DN, Chase MW. 1999. Application of ITS (nrDNA) sequences in the phylogenetic study of *Tyromyces* s.l. Mycological Research. 103: 219–229.

Zhao RL., Karunarathna S, Raspé O, Parra A, Guinberteau J, Moinard M, Kesel AD, Barroso G, Courtecuisse R, Hyde KD, Guelly AK, Desjardin DE, Callac P. 2011. Major clades in tropical *Agaricus*. Fungal Diversity. 51: 279–296. http://dx.doi.org/10.1007/s13225-011-0136-7